THE NATURE OF THE "PENTOSE" REACTION IN AQUEOUS EXTRACTS OF PSORIASIS SCALES*

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Increased content of pentose in the water-soluble components of psoriasis scales compared to normal skin scales was demonstrated with the orcinol reaction by Grüneberg and Szakall (1) and confirmed by Flesch and Esoda (2) and Wheatley and Farber (3). Similarly, increased pentose in post-sunburn scales was reported by Wheatley and Farber (4). However, Wheatley was unable to identify free pentose using highly sensitive paper chromatographic procedure (3).

We investigated the water-soluble fraction of psoriasis scales in an attempt to characterize the substance responsible for the positive test in the orcinol reaction. Scales obtained following sunburn and peeling were chosen as controls, both for the reported higher pentose content and for its ready availability at certain seasons.

METHOD

Aqueous extracts of psoriasis scales and non-psoriatic control skin obtained following sunburn and peeling were prepared by homogenizing 10 mg scales per 1 ml cold water for about 5 minutes, or longer if needed to obtain a fine suspension. The mixture was centrifuged and the supernatant liquid was removed for analysis. Extracts were stored at −4° C except when needed for analysis; then they were kept in ice. Orcinol reagent was prepared freshly each time before use as described by McRary and Slatery (5). To 0.10 ml aqueous extract was added 0.3 ml orcinol-$FeCl_3$-HCl reagent. A ribose solution used for standard contained 2.0 μg./0.1 ml. Total reducing sugars were determined by the Somogyi-Nelson method (6). Paper chromatographic determinations for sugars were carried out using the following solvents: butanol, pyridine, water (80–80–40: BuPy): buffered phenol-water (Ph): and butanol, glacial acetic acid, water (80–20–20: BuAc). Reagents used for detection of sugars were aniline-phthalic acid (7), orcinol (2% dissolved in 30% trichloracetic acid) and the Somogyi-Nelson total sugar reagents (6). Phosphorylated sugars and other organic phosphates were detected by spraying with ammonium molybdate, hydrochloric acid, perchloric acid in 85% ethanol and exposure to ultraviolet light (8).

RESULTS

A positive reaction was obtained on heating 0.1 ml aqueous extract of psoriasis scales with 0.3 ml of orcinol-$FeCl_3$-HCl reagent. The control skin likewise gave a faintly positive reaction. Total reducing substance in control sunburn skin was almost five times greater than in psoriasis scales. In Table I are shown the total reducing substances and apparent pentose in psoriasis scales and control skin. The values for pentose and total reducing sugars in psoriasis scales are in the same range as those reported by other investigators (3). Apparent pentose content of our post-sunburn skin was in the same range as that reported by other investigators for callus (32 ug/100 mg scales) (3) or for scrapings from apparently normal skin (38 ug/100 mg scales) (4).

Chromatograms were prepared using 0.1 ml and 0.250 ml of each aqueous extract. Standard sugars placed on the chromatogram as markers were glucose, galactose, fructose, lactose, ribose, xylose, and arabinose. Replicate chromatograms were resolved in each of the three solvent systems BuPy, Ph, BuAc. One was sprayed with aniline-phthalate and the other with 2% orcinol in 30% aqueous trichloracetic acid. After heating at 110° C for 20 minutes, the aniline chromatogram revealed brown spots with hexoses and lactose and red colors with pentoses. The minimum amount of sugar which can be detected on chromatograms is 2–4 μg. Figure 1 shows a typical chromatogram run in BuPy solvent. On the chromatogram sprayed with orcinol and heated at 110° C for 20 minutes, pentoses produced green or purple colors. Hexoses did not react. Substitution of trichloracetic acid for the hydrochloric acid used in the general test was necessary to prevent destruction of the paper during heating. No free sugar was detected with either reagent in the aqueous extract of psoriasis scales or normal skin. When no free sugars were detected

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TABLE I

Concentration of "Apparent Pentose" and Total Reducing Sugars in Aqueous Skin Extracts before and after Mild Acid Hydrolysis

	Apparent Pentose (as μg. ribose/100 mg. scales)		Total Reducing Sugars (as μg. glucose/100 mg. scales)	
	Before	After Hydrolysis	Before	After Hydrolysis
Psoriasis Scales	140	70	500	230
Non-psoriatic (sunburn) skin	40	23	2370	1915

to account for results in the total reducing sugar reaction, a chromatogram was sprayed first with the copper reagent, heated 20 minutes at 100° C and then with arsenomolybdate solution. A strong positive reaction at Rf .20 (BuPy) was obtained with normal skin. None of the sugar markers migrated to similar position. Psoriasis scales gave a smaller spot in the same region. The reducing substance did not correspond with any of the sugars used as standards.

Neither of the paper chromatographic reagents, aniline-phthalate or orcinol, is sensitive for bound sugars. However, heating for several minutes in the presence of concentrated hydrochloric acid, as described in the orcinol reaction, should be sufficient to liberate sugars bound to either phosphates, glycoproteins, or nucleosides. A 1 ml portion of the aqueous extract was made approximately 2N by addition of 0.2 ml concentrated hydrochloric acid. Each extract was sealed in a tube and autoclaved at 120° C for one hour. The hydrolysates were then tested with the orcinol-$FeCl_3$-HCl reagent and with the Somogyi-Nelson reagent. The apparent pentose content of the hydrolysate was approximately 50% of that found in the unhydrolysed aqueous extract of both psoriasis scales and control skin. A change in total reducing substances was also found. These results are summarized in Table I. Both orcinol and the Somogyi-Nelson reagent are general reagents for carbohydrates and other reducing substances. Some of these substances are decomposed by mild acid hydrolysis so that the reducing properties are no longer present. Thus the apparent content of pentose or reducing substances decreases. If the reactions were the result of free pentose or free hexose, acid hydrolysis should have no effect. Estimate of the glucose in the hydrolysates was made by comparison of the spots with known amounts of the sugar. The reducing substance responsible for the positive reaction with the Somogyi-Nelson reagent was not identified or examined further.

The non-specificity of the orcinol reaction, and the absence of detectable amounts of free pentose in the aqueous extracts led us to test for a number of substances other than sugars which have been reported or might occur in psoriasis scales. These included uracil, xanthine, hypoxanthine, guanine, chondroitin sulfate, glycogen, adenosine monophosphate (AMP), and uridine diphosphoglucose (UDPG). Some of these substances are shown on the composite chromatogram, Figure 1. Positive orcinol reactions in solution were obtained with chondroitin sulfate (green), adenosine monophosphate (green), glycogen (green-gray), and UDPG (green). Pentoses typically produce green colors in the reaction.

Guanine, glycogen and chondroitin sulfate gave positive reactions with the Somogyi-Nelson reagent. The aqueous extract gave a negative reaction for metachromatic staining with toluidine blue, a sensitive test for chondroitin sulfate.

Increased amounts of organic or "bound" phosphorus have been reported in psoriasis scales (3). Both adenosine monophosphate and uridine diphosphoglucose reacted with orcinol-$FeCl_3$-HCl to give green colors similar to pentoses. Chromatograms were then sprayed with the reagent for organic phosphates and exposed to ultraviolet light. Organic phosphate was detected in both control and psoriasis extracts. Migration characteristics of the organic phosphates together with marker substances are shown in Table II, and in Figure 1. UDPG migrated to positions similar to the organic phosphate spot in psoriasis scales. No organic phosphate spot was noted following mild hydrolysis, although a yellow spot appeared prior to exposure to ultraviolet light which is characteristic of inorganic phosphate. Both UDPG and AMP gave positive reactions for organic phosphate.

To clarify the origin of the glucose found in the hydrolyzed extracts, the aqueous extract of psoriatic scales was applied as a streak along a line one inch from the bottom of a sheet of

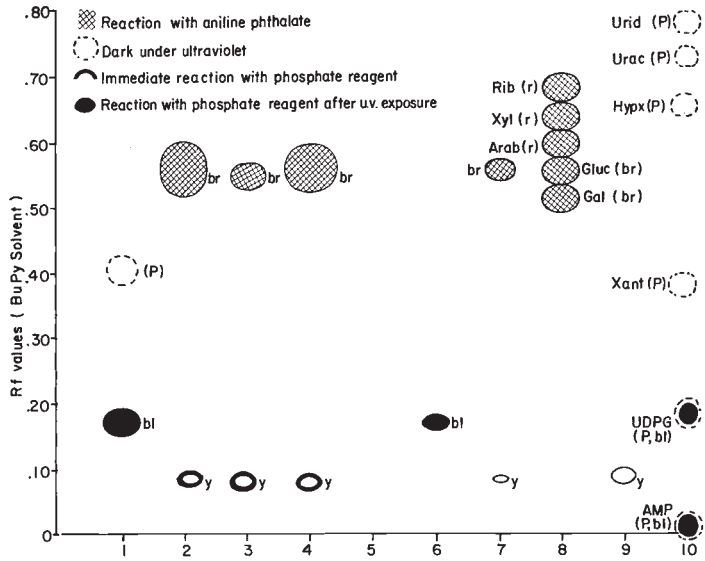

FIG. 1. Composite chromatogram of aqueous extract of psoriatic scales and control skin before and after mild acid hydrolysis, and standards of sugars, nucleic acid derivatives and phosphate. Solvent: Butanol-pyridine-water (80–80–40): 1. Aqueous extract of psoriatic scale (2.5 mg/250 μl); 2. Aqueous extract of psoriatic scale after mild acid hydrolysis (1 mg/100 μl); 3. Portion of chromatogram in spot between Rf .00 and .05 cut out, eluted, subjected to mild hydrolysis, then rechromatographed. (Prot. in text); 4. Portion of chromatogram in spot 1 between Rf .15 and .25 cut out, eluted, subjected to mild hydrolysis, then rechromatographed. (Phos. in text); 5. Portion of chromatogram in spot 1 between Rf .50 and .60 cut out, eluted, hydrolyzed (Rf .50–.60 in text); 6. Aqueous extract of sunburn skin (1 mg/100 μl); 7. Aqueous extract of sunburn skin after mild hydrolysis (1 mg/100 μl); 8. Sugar standards—10 μg of each: Rib = ribose, Xyl = xylose, Arab = arabinose, Gluc = glucose, Gal = galactose; 9. Sodium dihydrogen phosphate—15 ug; 10. Standards of nucleic acid derivative—10 μg of each: Urid = uridine, Urac = uracil, Hypx = hypoxanthine, Xant = xanthine, UDPG = uridinediphosphoglucose, AMP = adenosinemonophosphate.

Key to reactions: br: brown, aniline-phthalate; r: red, aniline-phthalate; p: purple) dark) absorption under ultraviolet light; bl: blue, phosphate reagent, after exposure to ultraviolet light; y: yellow, phosphate reagent, before exposure to ultraviolet light.

Whatman 3 mm paper. About 1 ml was applied to a single sheet. The chromatogram was resolved in BuPy solvent and a guide strip cut off and developed for organic phosphorus. Protein does not migrate in this solvent, so an area at the point of application of the sample (Rf .0–.05, Prot.), an area corresponding to positive organic phosphate (Rf .15–.25, Phos.) and an area near the center of the sheet (Rf .50–.60) were cut out and eluted with about 1 ml of water into small flasks. The eluate was made approximately 2 N with HCl, sealed, hydrolyzed one hour as described previously. The three separate portions were chromatographed. Glucose was found in both (Prot.) and (Phos.) with the larger concentration in the area eluted from the positive organic phosphate spot. On the chromatogram hydrolysate (Prot.) contained 15 μg glucose and hydrolysate (Phos.) contained 32 μg glucose. Sugar was not detected in the hydrolysate from (Rf .50–.60). These results are illustrated in Figure 1. The hydrolysates were then tested with the orcinol-$FeCl_3$-HCl reagent and the Somogyi-Nelson reagent. The hydrolysates were negative to the orcinol reagent; both (Prot.) and (Phos.) gave weakly positive tests for total reducing sugars.

DISCUSSION

Some of the glucose may be derived from a glycoprotein. It should be pointed out that the area of positive organic phosphate also contained the unknown reducing substance. While this substance was apparently unchanged by the mild hydrolysis of the total extract, there was insufficient material to examine the hydrolysates of eluates from the paper for the material.

Release of glucose by mild acid hydrolysis of a substance containing an organic phosphate bond together with 50% decrease in the apparent pentose content suggest that the "pentose" in psoriasis scales may actually be a complex

TABLE II
Organic Phosphates and Nucleic Acid Derivatives

	Rf Values			Reactions Ultraviolet Light	Phosphate Reagent	
	BuPy	Ph	BuAc		Immediate Reaction	Reaction after exposure to U.V. light
UDPG	.18	.03	.00	Dark	None	Blue
AMP	.00	.08	.12	Dark	None	Blue
Uracil	.74	.82	.51	Dark	None	None
Uridine	.77	.32	.19	Dark	None	None
Xanthine	.37	.65	.35	Dark	None	None
Hypoxanthine	.66	.90	.42	Dark	None	None
NaH_2PO_4	.03	.05	.15	None	Yellow	Blue
Unknown from psoriasis scales	.15	.05	.00	Interference	None	Blue
Hydrolysate of unknown	.03	.06	.15	Interference	Yellow	Blue

molecule containing both phosphate and glucose. Uridinediphosphoglucose is such a complex molecule. However, we have as yet no direct evidence that the apparent "pentose" in psoriasis scales represents uridinediphosphoglucose. Neither uridine, uracil, nor ribose was detected on chromatograms of the hydrolyzed material when examined under ultraviolet light, or after reaction with aniline or orcinol. The amount of material available for hydrolysis was small, and the breakdown products may have been present in concentration too low for detection. Therefore, UDPG (0.1 μm in 0.1 ml) was hydrolyzed for one hour in 2 N HCl as described for the aqueous skin extracts. Glucose was detected chromatographically in the hydrolysate of UDPG, although none was present prior to hydrolysis. The organic phosphate reaction was negative in hydrolyzed UDPG, although inorganic phosphate was detected. Again, neither uridine, uracil nor ribose was detected in the hydrolysate, indicating that the amounts were too small for chromatographic detection.

Uridine and uracil were detected in extracts of psoriasis scales by Wheatley and Farber and by Hodgson (9). It has been our experience that nucleotides and nucleosides, whether present in urine or tissue extracts or used as reagents in enzymatic studies, break down readily at room temperature. We have made a practice of working with solutions kept cold and stored in a frozen state. The above authors suggested that the observed abnormalities, in the composition of psoriasis scales, increased content of pentose, organic phosphate, and uracil, could be accounted for by accumulation of UDPG. The data presented here suggest that metabolism of UDPG in skin might be examined further for possible clues to the mechanism of the lesion in psoriasis and other scaling dermatoses.

SUMMARY

Aqueous extracts of psoriasis scales and nonpsoriatic sunburn skin were chromatographed in several solvents. Free reducing sugars could not be demonstrated on chromatograms using either aniline-phthalate or orcinol reagent.

Both psoriasis scales and control skin contained material which gave a positive reaction using orcinol-$FeCl_3$-HCl. Mild acid hydrolysis of the aqueous extract resulted in loss of approximately 50% of the orcinol-positive material. When the extract was subjected to mild hydrolysis glucose was detected in the hydrolysates, although none was demonstrated prior to hydrolysis. The amount of free glucose derived from hydrolysis of psoriasis scales was greater than that of the control skin. Organic phosphate was demonstrated in extracts of psoriasis scales in concentrations two to three times that of control skin. When the area on the chromatogram containing organic phosphate was eluted and hydrolyzed, glucose was released, and organic phosphate was converted to inorganic phosphate.

Substances other than pentoses which reacted with orcinol-$FeCl_3$-HCl to give green colors indistinguishable from pentose were adenosine monophosphate, chondroitin sulfate and uridine-

diphosphoglucose. Of these only uridinediphosphoglucose releases both glucose and inorganic phosphate on mild hydrolysis. While no direct evidence is presented, these data suggest that the "apparent pentose" in psoriasis scales may be a complex molecule similar to uridinediphosphoglucose.

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